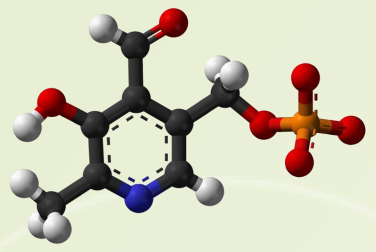

Comparing Vitamin B6 Concentrations in High Light Plants and Shade Tolerant Plants

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SOUTHEAST

Abstract

Vitamin B6 is an important cofactor in over 100 enzymatic reactions in the cell. One function of vitamin B6 is to neutralize reactive oxygen species. These can lead to cell damage at high concentration and tend to accumulate more quickly in high light. We hypothesize that high light plants will possess greater concentrations of vitamin B6 than shade tolerant plants. We collected plants from high light and shaded environments and are performing yeast assays to determine vitamin B6 content from plant extracts. We will compare the average vitamin B6 concentrations between high light plants and shade tolerant plants.

Background Information

Vitamin B6 is a coenzyme that regulates many essential cellular processes, including biosynthesis of amino acids, fatty acids, hormones, chlorins, and neurotransmitters. [1] It has also been demonstrated the vitamin B6 assists in stress tolerance via neutralization of reactive oxygen species (ROS). [1][2][3]

ROS are chemically reactive chemicals that contain oxygen. Metabolic processes often produce ROS as a byproduct, though under times of intense stress, ROS levels can rise to a point where they can cause cell damage. This state of cell damage due to ROS is referred to as oxidative stress. [2][3]

Exposure to high intensity light is one of the factors that can lead to oxidative stress. [2][3] Since vitamin B6 can quench ROS, it stands to reason that a higher concentration of vitamin B6 should be present in plants that are continually exposed to high levels of light than those that display shade-tolerance. This study was performed with the goal of determining if there is a significant difference in vitamin B6 concentration between high light plants and shade tolerant plants.

Methodology

1. Plant samples were collected from an open, well-lit field and from an area of shaded forest.

2. Vitamin B6 was extracted from plants by grinding plant tissue in 0.44M HCl and autoclaving samples at 121 °C for 4 hours. Samples were then treated with B-glucosidase and acid phosphatase.

3. Yeast samples were prepared; petri plates were inoculated with yeast and incubated at 30°C. Plates were retrieved and yeast transferred to conical tubes in aqueous solution. Yeast solution was washed, then stored and refrigerated.

4. Dilutions were created containing a ratio of 2 ul of yeast solution to 998 ul of water. Yeast was counted under a microscope by transferring dilution into hematocytometer.

5. Test tubes were prepared for yeast assay. Each tube was filled with 3 ml of pyridoxine Y media and a volume of yeast containing approximately 5 billion cells. Vitamin B6 extract was added to standard tubes, while plant extract was added to experimental tubes. Tubes were then incubated at 30°C for 24 hours.

6. 1 ml of yeast solution from each tube was transferred to a corresponding cuvette. Absorbance rating of samples was determined using a spectrophotometer.

7. Absorbance rating data was used to establish a linear regression equation based on a standard curve. The average vitamin B6 Content of each sample group was then calculated. A T-test was performed to analyze the difference between the sample groups.

Figure 1. Plant Sample Collection.
After being retrieved from the environment, samples were frozen in liquid nitrogen for long term storage.

Results

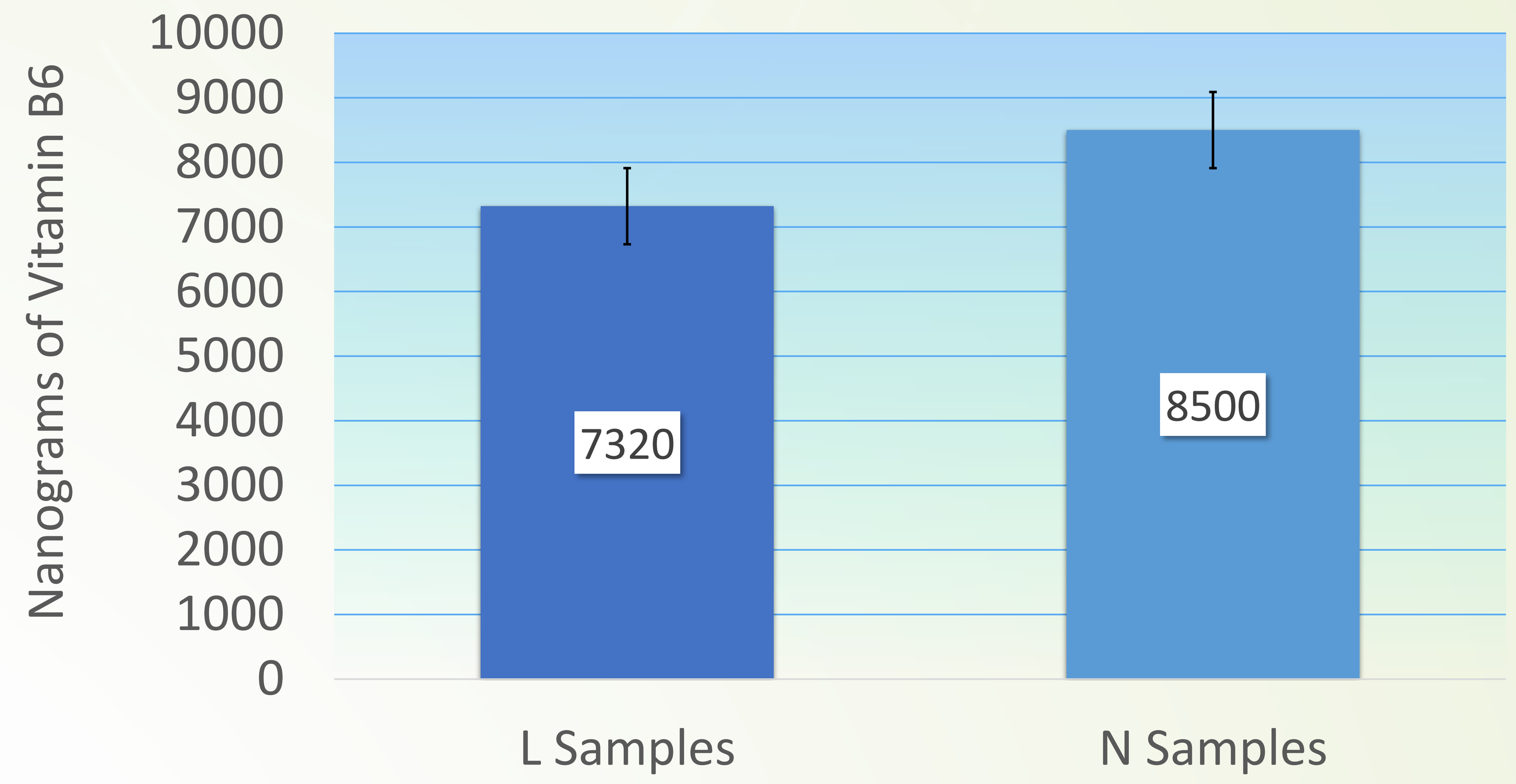

Figure 2. Average Nanograms of Vitamin B6 per Gram of Leaf Tissue

L samples are taken from *Melilotus albus* (common name, white sweetclover). N samples comprise tissue from *Rudbeckia hirta* (black-eyed Susan). Statistical analysis was performed via a two-tailed t-test of two sample sets with unequal variance. Results indicate that there was not a significant difference in vitamin B6 concentration between the two sample groups; $t(42) = -0.834$, $p = 0.409$, $\alpha = 0.05$.

Conclusion

- Statistical analysis revealed that there is not a significant difference between the vitamin B6 concentration of the L and N sample groups.
- The samples tested in this study were both taken from high-light plants; additional samples, including those from shade tolerant plants, must be assayed to provide meaningful data to support or refute the hypothesis.

References

1. Parra, Marcelina, et al. "Vitamin B6 and Its Role in Cell Metabolism and Physiology." Cells, vol. 7, no. 7, 22 July 2018, p. 84, 10.3390/cells7070084.
2. Czégény, Gyula, et al. "Multiple Roles for Vitamin B 6 in Plant Acclimation to UV-B." Scientific Reports, vol. 9, no. 1, 4 Feb. 2019, pp. 1–9, www.nature.com/articles/s41598-018-38053-w, 10.1038/s41598-018-38053-w.
3. Havaux, Michel et al. (2009). Vitamin B6 deficient plants display increased sensitivity to high light and photo-oxidative stress. BMC plant biology. 9. 130. 10.1186/1471-2229-9-130.

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